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Association between Respiratory Disease and Bacterial and Viral Infections in British Racehorses

J. L. N. Wood,* J. R. Newton, N. Chanter,† and J. A. Mumford

Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, United Kingdom

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Respiratory disease is important in horses, particularly in young Thoroughbred racehorses, and inflammation that is detected in the trachea and bronchi (termed inflammatory airway disease [IAD]) is more significant in this population in terms of impact and frequency than other presentations of respiratory disease. IAD, which is characterized by neutrophilic inflammation, mild clinical signs, and accumulation of mucus in the trachea, may be multifactorial, possibly involving infections and environmental and immunological factors, and its etiology remains unclear. This 3-year longitudinal study of young Thoroughbred racehorses was undertaken to characterize the associations of IAD and nasal discharge with viral and bacterial infections. IAD was statistically associated with tracheal infection with *Streptococcus pneumoniae* (capsule type 3), *Streptococcus zooepidemicus*, *Actinobacillus* spp., and *Mycoplasma equirhinis* and equine herpesvirus 1 and 4 infections, after adjustment for variation between training yards, seasons, and age groups. The association with *S. pneumoniae* and *S. zooepidemicus* was independent of prior viral infection and, critically, was dependent on the numbers of organisms isolated. *S. pneumoniae* was significant only in horses that were 2 years old or younger. The prevalence and incidence of IAD, *S. zooepidemicus*, and *S. pneumoniae* decreased in parallel with age, consistent with increased disease resistance, perhaps by the acquisition of immunity. The study provided evidence for *S. zooepidemicus* and *S. pneumoniae* playing an important etiological role in the pathogenesis of IAD in young horses.

Respiratory disease is common in racehorses in training (10, 15, 29), as it is in most young domestic animals species. Both the incidence and prevalence of respiratory disease in racehorses vary widely between yards and between years (38, 46, 58), but the reasons underlying this variation, including the causes of the disease, are not defined.

Inflammatory lower airway disease (IAD), which is usually associated with increased amounts of mucus visible in the trachea after exercise and increased proportions of inflammatory cells (44), is common in young racehorses around the world, despite marked differences in management and climate. Clinical signs associated with IAD include coughing (10, 16) and poor performance in racing (30, 37, 44, 45). IAD is considerably more common than signs of upper respiratory disease and in young racehorses has a mean monthly prevalence of around 12% and an incidence of around 10 cases/100 horses/month (10, 58). The mean duration of each incident is around 8 weeks, and the disease is often recurrent in individuals. The monthly prevalence and incidence of signs of upper respiratory disease are around 5% and 5 cases/100 horses/month (10, 38, 58).

The etiology and pathogenesis of IAD are poorly defined, but the etiology is probably multifactorial (10, 44). Studies focusing on individual agents suggest the possible involvement of viral infection (59), bacterial infection (10, 14, 16, 42, 43, 56), and environmental loading of the respiratory system (10, 27, 33, 50), as well as dysregulation of inflammatory processes (6). Individual bacteria commonly reported from cases include *Streptococcus zooepidemicus* (10, 14, 16, 42, 56), *Streptococcus pneumoniae* (14, 16, 56), *Actinobacillus* spp. (14, 16, 42, 56), and *Mycoplasma felis* (42, 55). However, few investigations have attempted to identify by multivariable epidemiological techniques those factors most likely to be of significance, which would provide a measure of those that deserve more detailed etiological investigations.

We undertook a large-scale longitudinal study of respiratory disease in racehorses over 3 years in seven different training yards in an attempt to define the relative importance of all of the infections associated with IAD and nasal discharge (ND). The specific aim of the study was to identify those agents most strongly quantitatively associated with disease and to assess whether their prevalence and incidence were sufficient to indicate an important role in etiology, having taken account of multiple independent sources of variation.

MATERIALS AND METHODS

Data. Horses were selected from the British flat racing population in a convenience sample of seven different training yards and were studied over a 38-month period from November 1993 to December 1996. Ten to fifteen horses were selected in each yard. A convenience sample was necessary due to the high level of cooperation required from racehorse trainers. The study population comprised 148 horses that were observed for a total of 1,604 months.

Horses were clinically examined on a monthly basis. An unguarded wire-mounted gauze swab was collected from the nasopharynx, and clotted blood was collected for viral serology immediately after exercise. An endoscopic examination of the trachea to the level of the carina was then performed (7, 32), with a tracheal wash sample being collected transendoscopically by instilling 30 ml of sterile phosphate-buffered saline into the distal trachea and then immediately aspirating it.

* Corresponding author. Mailing address: Epidemiology Unit, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 0NG, United Kingdom. Phone: 44 1638 750659. Fax: 44 1638 666659. E-mail: james.wood@aht.org.uk.

† Present address: Intervet Plc, Walton Manor, Milton Keynes, Bucks, MK7 7AJ, United Kingdom.

Laboratory examinations. Evidence of viral infection (including influenza H7N7 and H3N8, equine herpesvirus-1 [EHV-1] and EHV-4, equine rhinovirus-1 [ERV-1] and ERV-2, and equine adenovirus) was assessed through serological examination of serial blood samples by using complement fixation tests for EHV and ERV (49) and hemagglutination inhibition tests for influenza and adenovirus (4). Tracheal wash samples and nasopharyngeal swabs were returned to the laboratory on blocks of ice and assessed by standard quantitative cultural methods for the presence of bacteria, including mycoplasma (42).

Definitions of disease. IAD was defined on the basis of a cumulative score composed of (i) visual assessment of the amount of mucus in the trachea, scored from 0 to 3 (7); (ii) cytological assessment of the degree of neutrophilic inflammation in tracheal aspirates, based on the proportion of neutrophils in the cellular population (47, 54); and (iii) the number of nucleated cells per cubic millimeter of tracheal wash. The degree of inflammation was scored on an ordinal scale, from 0 to 3, and IAD was defined as an inflammation score of ≥ 2 out of 3. A score of 3 out of 3 was derived from scoring ≥ 2 out of 3 on the amount of mucus in the trachea, and having moderate or greater proportions of neutrophils in the tracheal wash and $\geq 1{,}000$ nucleated cells/mm^3 of tracheal wash.

ND was defined as the presence of a mucopurulent or serous nasal discharge at the time of clinical examination. ND was not recorded in one training yard, which was excluded from relevant analyses.

Statistical methods. We undertook statistical analyses to determine the infections associated with the disease outcomes. Initially, data were explored with simple descriptive and categorical statistical approaches (1). Univariable association between categorical variables and disease was determined by using standard chi-square analyses and between continuous variables and disease by using *t* tests, assuming unequal variances. The functional form of the relationships between continuous variables, including $\log_{10}$ transformed bacterial counts (CFU/ml + 1), and disease was explored graphically. Univariable ordinary logistic regression analysis was used to identify the most parsimonious fitting variable form for each of the continuous and categorical variables.

Following univariable data analysis, multivariable mixed-effects logistic regression (MELR) models of the longitudinal data set were developed by using a forward stepwise approach. Random effects to account for horse-level effects were included in final models to account for lack of independence of repeated observations from the same individual (20). Autocorrelation variables were developed to adjust models for the effects of disease in the previous observation period, and single period time-lagged variables were tested in models to determine the effects of infections (viral and bacterial) in previous time periods (18). Statistical analyses were undertaken with SAS (version 8.02; SAS Institute Inc., Cary, N.C.) and Egret (Cytel Software Corporation, Cambridge, Mass.).

Variables associated with disease in univariable analyses ($P < 0.4$) were tested sequentially in regression models, and those that were significantly associated with the outcome (Wald χ^2, $P \leq 0.05$) or whose inclusion in the model was associated with a significant reduction in model deviance (likelihood ratio χ^2 test, $P \leq 0.05$) were retained in the models (28). Biologically meaningful two-way interaction terms between significant variables were also included if they provided a significant reduction in model deviance (likelihood ratio χ^2 test, $P \leq 0.05$). After inclusion of such interaction terms, the model-building process was repeated to check that significant variables had not been excluded. The quality of model fit was assessed by using the Hosmer-Lemeshow statistic (28). Levels of statistical significance were generally set at $P \leq 0.05$, although terms significant in ordinary logistic regression models were generally retained in mixed-effects models if they were associated with improved MELR model fit. The effects of marginally significant variables ($0.05 < P < 0.1$) were evaluated in terms of their impact on the quality of model fit through the Hosmer-Lemeshow test, particularly given the substantially reduced power of studies to detect significant interaction terms (48). Caterpillar plots (21, 22) were used to examine the distribution of horse-level residuals, including those for outliers, following development of MELR models.

RESULTS

IAD. Univariable and other exploratory analyses (58) demonstrated that the odds of having IAD significantly decreased with the age of the horse, varied substantially between training yards and between seasons, decreasing through the year from a peak in the early spring, and generally increased significantly with the presence of specific species of bacteria isolated from tracheal wash samples (Table 1). The strongest associations were between IAD and *S. zooepidemicus*, *S. pneumoniae*, *Actinobacillus* and *Pasteurella* spp., and *Mycoplasma equirhinis* (Table 1). There was significant correlation between the presences of these bacterial species, consistent with clinical observations that mixed infections in the presence of disease were common. No bacterial growth was detected in 22% of tracheal wash samples. The associations between the presence of the infection in the previous time period and IAD were weaker than those for the presence of the bacteria at the time of sampling. The only viral infection significantly associated with IAD was EHV. Graphical exploration suggested that, for the bacteria associated with disease, the association between numbers isolated and IAD was linear on the logarithmic scale.

TABLE 1. Univariable associations between disease (IAD and ND) for different infections

Infection or seroconversion	*t* test *P* value[a]			
	IAD		ND	
	This month	Previous or next month	This month	Previous or next month
Infection				
S. zooepidemicus	<0.0001	<0.0001	0.1	0.5
S. pneumoniae	<0.0001	0.003	0.4	0.2
Pseudomonas aeruginosa	0.8		0.04	
Pseudomonas spp. (non-*aeruginosa*)	0.06		0.8	
Acinetobacter spp.	0.003	0.07	0.01	0.07
Enterobacter spp.	0.5		0.4	
S. equisimilis	0.7		0.9	
Escherichia coli	0.01	0.2	0.9	0.9
Coagulase-negative *Staphylococcus* spp.	0.01	0.9	0.8	0.5
Bacillus spp.	0.9		0.9	
Nonhemolytic *Streptococcus* spp.	<0.0001	0.0004	0.1	0.8
Serratia spp.	0.05	0.6	0.001	0.2
Moraxella spp.	0.7		0.9	
Klebsiella spp.	0.3		0.9	
Bordetella bronchiseptica	0.5		0.9	
Actinobacillus and *Pasteurella* spp.	<0.001	0.0001	0.1	0.9
M. felis	0.07	0.03	0.5	0.4
M. equirhinis	<0.0001	0.02	0.9	0.6
Non-*M. felis*, slower-growing glucose fermenter	0.08	0.3	0.08	0.9
"D87-like" isolates	0.3	0.1	0.001	0.003
Other *Mycoplasma* spp.	0.1	0.3	0.02	0.3
Viral Seroconversion				
Influenza A/equi-2	0.4	0.6	0.0002	
Equine herpesvirus (1 and 4)	0.9	0.003	0.8	0.08
Equine rhinovirus-1	0.8	0.1	0.07	0.3
Equine rhinovirus-2	0.1	0.2		0.4
Equine adenovirus	0.4	0.2		0.7

[a] *P* values for bacterial infection are given for a month and the previous month, while *P* values for viral seroconversion are given for a month and the next month.

Univariable MELR modeling showed that there was significant aggregation of disease within horses ($P < 0.001$) and that it was essential to consider random-effects terms to account for the repeated observations from each horse.

The final mixed-effects multivariable model contained terms

TABLE 2. Multivariable MELR model of variables significantly associated with IAD

Variable	β	SEβ	*P* value[a]	Odds ratio	95% CI[b]
Intercept (α)	−3.75	0.76	<0.0001		
Infective variables					
S. zooepidemicus count	0.48	0.14	0.0007	1.62	1.24–2.13
Actinobacillus spp. count	0.68	0.14	<0.0001	1.97	1.49–2.60
S. zooepidemicus × *Actinobacillus* spp.	−0.07	0.049	0.15	0.93	0.85–1.02
S. pneumoniae count	0.34	0.14	0.016	1.41	1.07–1.85
S. pneumoniae × age ≥3 years	−0.91	0.41	0.029	0.40	0.18–0.90
M. equirhinis detected	1.33	0.54	0.014	3.80	1.33–10.84
M. equirhinis × *Actinobacillus* spp.	−0.50	0.20	0.016	0.61	0.41–0.91
Acinetobacter spp.	0.40	0.16	0.011	1.50	1.10–2.04
EHV seroconversion next month	1.58	0.65	0.016	4.86	1.36–17.34
Trainer			0.0014[c]		
1	Referent				
2	−0.48	0.76	0.53	0.62	0.14–2.74
3	−0.93	0.83	0.26	0.39	0.08–1.99
4	0.83	0.77	0.28	2.30	0.50–10.50
5	−0.81	0.81	0.32	0.44	0.09–2.19
6	1.45	0.80	0.071	4.25	0.89–20.22
7	0.19	0.85	0.83	1.21	0.23–6.37
Age code			0.028[c]		
≤1 year	2.65	1.25	0.037	14.09	1.21–164.26
2 years	Referent				
3 years	−0.81	0.39	0.037	0.44	0.21–0.95
≥4 years	−1.77	0.77	0.023	0.17	0.04–0.77
Season			0.024[c]		
Nov. to Jan.	Referent				
Feb. to Apr.	0.72	0.49	0.15	2.05	0.78–5.39
May to July	0.26	0.50	0.59	1.30	0.49–3.45
Aug. to Oct.	−0.76	0.55	0.17	0.47	0.16–1.39
Disease in previous month	0.65	0.36	0.076	1.91	0.94–3.89
Random effects variance	1.14	0.59	0.058[a]		

[a] *P* values are from the Wald χ^2 test.
[b] 95% CI, 95% confidence interval of the odds ratio.
[c] Likelihood ratio statistic.

for the numbers of *S. zooepidemicus*, *Actinobacillus* spp., *S. pneumoniae*, and *Acinetobacter* spp. organisms and for the detection of *M. equirhinis* and EHV seroconversions (Table 2). The model also contained terms for the horses' ages, the trainer, and the season, as well as an autocorrelation term representing disease in the previous month. There were significant interactions between the numbers of *S. zooepidemicus* and *Actinobacillus* spp. organisms, between the presence of *S. pneumoniae* and whether a horse was 2 years old or younger, and between the numbers of *Actinobacillus* spp. organisms and the presence of *M. equirhinis*. The model described the data well (Hosmer-Lemeshow test, $\chi^2_{df=8} = 8.0$, $P = 0.43$). During model building, the interaction term between *S. zooepidemicus* and *Actinobacillus* spp. significantly reduced model deviance, and the autocorrelation term for disease in the previous month was also highly significant ($P = 0.0004$) prior to inclusion of the random, horse-level term. Removal of either term from the final model reduced the quality of model fit (Hosmer-Lemeshow test, $P = 0.26$ to 0.28). Horse-level residuals fell within 1 standard deviation of the mean for 138 of 148 horses.

The model showed that the odds of having IAD significantly increased with the numbers of *S. zooepidemicus*, *Actinobacillus* spp., and *M. equirhinis* organisms. There was a strong association between numbers of *S. pneumoniae* and IAD in horses that were 2 years old or younger but no significant association in older animals. The odds of having the disease decreased as horses grew older and varied significantly between training yards and between seasons, being most likely from February to April and least likely from August to September. The odds of having the disease were also increased if the horse had been affected in the previous month, consistent with the average duration of disease being around 2 months (58).

ND. The odds of having ND significantly decreased with age, in particular comparing yearling horses to 2-year-old animals (58). Univariable analyses suggested that the disease might vary with season ($P = 0.06$) and year ($P = 0.02$), but these terms were not significant in multivariable models. The detection of individual species of bacteria in the nasopharynx was not associated with ND, with the exception of *S. zooepidemicus* ($P = 0.02$). The univariable association between the detection of individual species of bacteria in tracheal wash samples and ND was not as strong as for IAD, with only a few species significantly associated with ND. Influenza (H3N8) seroconversion was significantly associated with ND in univariable analyses.

In the multivariable mixed-effects model, only age, the presence of IAD, and the detection of influenza (H3N8) and the serologically unidentified glucose-fermenting *Mycoplasma* sp. were associated with ND (Table 3). There was no evidence of clustering of the disease at the horse level ($P = 0.5$), and

TABLE 3. Multivariable MELR model of variables significantly associated with upper respiratory disease (ND)

Variable	β	SEβ	*P* value[a]	Odds ratio	95% CI[b]
Intercept (α)	−1.40	0.61	0.022		
Age					
≤1 year	Referent				
2 years	−2.49	0.66	<0.001	0.08	0.023–0.30
≥3 years	−2.50	0.66	<0.001	0.08	0.023–0.30
Presence of IAD	1.50	0.35	<0.001	4.46	2.26–8.80
Infective variable					
Influenza (H3N8) seroconversion	4.99	1.19	<0.001	146.3	14.17–1,511
Non-*M. felis*, slower-growing glucose fermenter	0.69	0.28	0.014	1.99	1.15–3.46
Random effects variance	1.2×10^{-11}	0.46	0.500[c]		

[a] *P* values are from the Wald χ^2 test.
[b] 95% CI, 95% confidence interval of odds ratio.
[c] One-sided test.

inclusion of a horse-level random effect had no effect on fixed-effect parameter estimates.

DISCUSSION

Despite the fact that IAD is one of the more common diseases affecting horses (44), previous work has identified potential etiological factors but left unanswered questions of the relative importance of bacterial and viral pathogens. The results presented here, and the description of incidence and prevalence rates of disease presented elsewhere (58), give a unique insight into IAD by providing considerably stronger evidence of characteristics of IAD only hinted at by previous smaller studies (9, 10, 14, 16, 42, 56, 60) and by identifying the most common and significant potential pathogens.

The sample of trainers may have influenced the precision with which the findings are generalizable to training thoroughbreds in general, but the intensity and length of commitment needed in a longitudinal study could only come from highly motivated individuals, and the prospective study design would in itself reduce bias. The mean duration of IAD incidents of around 2 months (10, 58) suggested that most IAD incidents will have been detected, but we recognize that the monthly sampling interval dictated by practical constraints could have missed some short episodes.

Temporal, spatial, and other correlations must be accounted for in longitudinal analyses (18, 62). The data set was not large enough for a monthly term, but the season variable, along with an autocorrelation variable representing the presence of disease in the previous month (62), addressed temporal correlations. The mean duration of IAD of around 2 months is entirely consistent with the finding that an autocorrelation term for disease in the previous month was close to significant ($P = 0.076$). Within-training-yard correlation was addressed by including a fixed-effect trainer variable in the IAD model.

A clear case definition for IAD was used, based on a combination of gross endoscopic evaluation of tracheal mucus and the presence of increased proportions of inflammatory cells, particularly neutrophils (44, 47), that has been used in many other studies (10, 47, 52, 54–58, 60). There are differences between this definition and others, and so care needs to be taken when comparing this study with other studies (15). IAD was associated with the occurrence of ND, although this might have been expected, as some tracheal mucus tends to appear at the nostrils after exercise.

The odds of having IAD varied substantially between horses, as shown by the size of random-effects variance. IAD was very common, particularly in 2-year-old horses, and the cumulative annual prevalence of IAD in this age group was 80% (58). Reasons for variations in disease frequency between horses are not understood but may reflect immunological and genetic factors. The chronic disease seen in some horses, particularly 2-year-olds affected with IAD for more than two-thirds of the year, is likely to include increased airway reactivity (26) and dysregulation of inflammation (6), although this possibility needs further investigation. Genetic factors may also influence susceptibility to IAD, as found with another equine bacterial lung disease (39).

The odds of having IAD varied significantly between trainers and between seasons, as did the prevalence of disease (58). The reasons for this result were not entirely clear but were not explained by the variation in the rates of the different infections detected. However, the pathogenicity of different subtypes of *S. zooepidemicus* might vary, as has been indicated for different *Actinobacillus* spp. (52), and different strains may have infected different training yards. Environmental factors are important in the etiology of IAD (33) and are likely to be responsible for at least some of the variation seen between trainers and years (58). A previous study found that the mean duration of IAD incidents was significantly higher for horses kept in an environment where dust loading was higher and ventilation was less well regulated (10). Respirable endotoxin, measured at the level of horses' nares, has been shown to be correlated with levels of neutrophilic inflammation in similar racehorses in Australia (31), and stabling in itself can cause some inflammation in airways (27, 50). However, direct comparison of these factors was outside the objectives of this work.

This work demonstrated a significant reduction in the odds of having IAD with age, as for incidence and prevalence (58). In the case of IAD, the decrease was continuous from 1-year-old to 3-year-old horses. The decrease in ND was most obvious

between yearling animals and 2-year-olds. In this population of horses entering training around the end of their second year, it was impossible to differentiate the effects of age from those of time in training (15, 16, 42). The reduction of rates of IAD with age suggests the development of resistance or immunity and is consistent with IAD in young racehorses having an infectious component to its etiology. This reduction is in contrast to the prevalence of reactive airway obstruction associated with allergy to molds and spores, which increases with age (19).

Bacterial isolates were not obtained from 22% of tracheal washes, and the prevalence of IAD in horses with no bacterial growth was 4%. In stark contrast, there was 80% prevalence of IAD in horses with $\geq 10^5$ CFU of bacteria/ml. The clear association of IAD with bacteria cultured from the trachea rather than the nasopharynx suggests that little part was played by any potential tracheal contamination by the endoscope. This finding is further supported by the lack of detectable bacteria in the majority of tracheal samples from animals free of IAD in spite of the almost invariable presence of nasopharyngeal commensals (43).

Consistent with other studies of the role of viruses in respiratory disease in racehorses (16, 42), there was little evidence of a significant role for any virus in IAD. EHV, the only virus associated with IAD, was detected in only 5% of time periods where IAD was detected and only 5% of time periods in which IAD was incident. Influenza (H3N8) infection, a well-established cause of upper respiratory disease in the horse (38, 40), was associated with ND, probably due to vaccine breakdown occurring, particularly in the young racehorse population (41).

The odds of having IAD were much increased in 2-year-old horses infected with *S. pneumoniae*, which was detected in 23% of such cases, but the infection was not associated with IAD in older animals. In contrast to the situation in humans (35), only one capsule type (type 3) has been reported from the horse (3, 56). The association with equine IAD exists across a wide range of studies (8, 16, 34, 56), and despite the fact that the genes for pneumolysin in the equine strain have been shown to be disrupted (53), lung disease in the horse can be experimentally reproduced with *S. pneumoniae* (5). The bacterium meets all of the criteria suggested for assigning causation of disease (25). That only capsule type 3 is found in horses and that the incidence of infection dramatically decreases with age are consistent with *S. pneumoniae* playing a significant role in IAD in young horses and with immunity being acquired, usually during the first year of exposure in training yards.

In contrast to *S. pneumoniae*, the strong association between *S. zooepidemicus* and IAD was not confounded by the age of the horse, although the prevalence and incidence of this infection do decrease with age, in parallel with those of IAD (58). *S. zooepidemicus* is a significant secondary pathogen in the horse (12), and it was isolated from the trachea of 66% of horses with IAD, with samples in 30% of cases having $\geq 10^3$ CFU/ml. Disease (51), including pneumonia (61), has been reproduced experimentally in the horse, although we are unaware of any reports in the literature of experimental reproduction of IAD. *S. zooepidemicus* has homologues of the *S. pneumoniae* PsaA protein (23) as well as many other virulence determinants shown to be important in other bacterial species (24). The genome of *Streptococcus equi* has a large number of virulence determinants that are also in group A *Streptococcus pyogenes* and that have been previously characterized (17), and as *S. equi* and *S. zooepidemicus* are so similar, this is likely to be true for *S. zooepidemicus* as well. Many different M protein types of *S. zooepidemicus* (36) exist, differentiated by opsonizing antibodies in rabbits. These can also be differentiated by their 16S-23S RNA intergenic spacer (13), although neither approach was used here. However, isolates from this and other, similar studies have been stored; analysis of the patterns of infection of different isolates differentiated through their intergenic spacers and M protein hypervariable regions is underway (J. R. Newton, N. Chanter, and J. L. N. Wood, unpublished data). Type-specific immunity to different types of *S. zooepidemicus* may develop in the horse, and the time required for horses to be exposed to the most common types would explain the difference after 1 year in training between the 30% reduction in age-specific incidence of *S. zooepidemicus* and the corresponding >50% reduction for *S. pneumoniae* (58).

Horses experience many different group C streptococcal infections, both commensal and pathogenic, which serologically cross-react to a considerable degree. The different types of *S. zooepidemicus* have even more cross-reactive antigens. There was no serological test available that could easily distinguish between an *S. equi*, *S. zooepidemicus*, or *Streptococcus equisimilis* isolate, let alone the different types of *S. zooepidemicus*. Serological investigations would yield valuable information but await the development of tests to specifically quantify antibody responses to the different types of *S. zooepidemicus*. Presently, the available serological tests would be difficult to interpret. Future specific tests might be based on the variable regions of the surface M-like protein (36) or other antigens that may be associated with types determined genetically (13).

As for *S. zooepidemicus*, we found *Actinobacillus* spp. to be both common (58) and closely associated with IAD. The methods that we used to differentiate *Actinobacillus* and *Pasteurella* spp. turned out to be insufficiently discriminatory to identify the isolates to species level (52). It is likely that the strength of association of each species with IAD would have varied (52). An association between these bacteria and respiratory disease occurs in other species and has been reported for the horse as well (14, 16, 56). The negative statistical interaction with *S. zooepidemicus* (Table 2) probably just reflects the correlation between the isolation of the organisms. Also, as for *S. zooepidemicus*, the reduction in incidence of infection with *Actinobacillus* spp. with the age of the horse (58) is consistent with the development of immunity to different species or types and may also be a reason underlying the reduction in frequency of IAD with age.

The few reported studies of the role of mycoplasma in equine respiratory disease give only rates or incidents of isolation (2). The most recent study of their role in equine respiratory disease failed to isolate any mycoplasma (16), although they were common in horses in Ontario (11). Despite *M. felis* having been associated with acute respiratory disease (42, 55), it was relatively uncommon in the sampled population. In contrast, mycoplasma infections, particularly *M. equirhinis*, were common in racehorses in Britain and were associated with IAD, although their role in pathogenesis remains unclear.

The patterns of epidemiology and apparent persistence of the viruses and bacteria found to be associated with disease in this study vary markedly. While for EHV, a large proportion of

the adult horse population are latently infected, equine influenza is an immunizing infection cleared within a maximum of 7 to 14 days, depending on the animal's immune status (38). Similar variation is likely to exist between the bacterial pathogens studied here but has not been so well characterized.

Even though 59% of horses with IAD had $\geq 10^3$ CFU of either or all of *S. zooepidemicus*, *Actinobacillus* spp., or *S. pneumoniae* organisms/ml isolated from trachea wash samples, detailed statistical exploration did not reveal any evidence for biological interaction between these species of bacteria. There are other possible causes of IAD in populations of young horses (16, 31), but the data presented above suggest that *S. zooepidemicus*, *Actinobacillus* spp., or *S. pneumoniae* may play a significant role in most cases.

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